NUTRITIONAL FACTORS IN HOST RESISTANCE

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To discuss the role of nutritional factors in host resistance is both a challenge and a frustration—a challenge because it is a complex and large assignment, and a frustration because the subject introduces the necessity of examining still further some basic concepts that have been employed throughout the present symposium. At the risk of incompleteness it will be necessary to concentrate on only a few items which have a special relevance to the preceding discussions, and in so doing to maintain the focus on what the organizers of this symposium had in mind as the main target.

It must be remembered at the outset that by shifting our attention to host nutrition we are raising our eyes from the detailed examination of microscopic and submicroscopic events in the host's organs, tissues, cells, and molecular processes to a setting on a different level in which our unit of study is the host in an environment, in particular in a nutritional environment. And we are now inquiring whether there are known manipulations of the nutritional environment which will affect the outcome of the collision of two species, the pathogen and the host. In phrasing the inquiry in this fashion emphasis is given the fact that we are thereby formally satisfying the specification that we are now standing with both feet in a specially recognized area of biology, namely, ecology. If nutrition is concentrated upon, it is always with the clear recognition that nutrition is but a part of the environmental concerns which are properly considered as a branch of ecology, that science dealing with the mutual relations between organisms *and their environment.* This recognition has certain heuristic values and one of these should be made explicit at the outset. It is simply that the science of ecology recognizes the cardinal importance of the genetic structure of the interacting populations it seeks to understand. The program, perhaps, has paid insufficient attention to genetics, although Dr. Gowen's subsequent discussion may tend to redress this imbalance. No theoretical outlook on resistance to infection can pretend to completeness which does not incorporate genetic analysis. That statement may or may not be treason, but it is a matter for regret that there is not time here to make the most of it.

In discussing nutritional factors in host resistance a review of previous reviews will not be made but attention will be called to recent developments which provide some fresh insights into general problems of natural resistance. Two such developments will be critically examined. One of these is the relation of protein nutrition to resistance to infection as exemplified by the experiments of Dubos and Schaedler (1–3), and the other concerns some of the lessons learned from our own experiences in the study of natural resistance to salmonellosis.

Dubos and Schaedler have advanced the thesis that the plane of protein nutrition, quantitatively and qualitatively manipulated, influences resistance to infection and that a lowered plane reduces resistance. This claim has been advanced on the basis of experiments with young mice using diets with protein contents ranging from 5 to 20 per cent and with four infectious agents, *Mycobacterium bovis*, *Mycobacterium fortuitum*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* type C. This set of observations seemingly fulfills the operational definition of a nutritional resistance factor which was proposed some 9 years ago (4). In proposing that definition an appeal was made to the unambiguous operations of adding or withholding nutrients to separate clearly what was believed to be the biologically separate attributes of resistance and susceptibility. These operational definitions were as follows: "When experiment shows us that withholding a nutrient increases the effect of an infection, and supplying the nutrient decreases the effect, then we can say that the given nutrient is a resistance factor, and what we have thereby affected is the character 'resistance'." And let it not be forgotten than in the phenomenology of nutrition there are susceptibility factors as well, and these are operationally definable as follows: "When experiment shows us that withholding a nutrient decreases the effect of an infection, and supplying the nutrient increases the effect, then we can say that the

given nutrient is a susceptibility factor, and what we have thereby affected is the character 'susceptibility'."

Now the role of protein as a resistance factor has been advanced, and denied, before. Indeed, in the experiments of Hill and Garren (5) on fowl typhoid, protein has been advanced as a susceptibility factor. Ruebner and Bramhall (6) reported that low protein intake lowered resistance of mice to mouse hepatitis virus. Sprunt and Flanigan (7) have advanced the most complicated hypothesis of all, and believe that protein deficiency is cyclic in its effects on resistance to viral disease, leading first to susceptibility, then to resistance, and thence to susceptibility again. It is unreasonable, however, to think that we will resolve this issue by confronting one claim with its counterclaim and finally calling for a show of hands. It may be that the confusion springs from some unanalyzed considerations and since these involve some issues of general significance, it is worthwhile to undertake an analysis of this problem.

As we survey the phenomenology of the Dubos-Schaedler experience, there is one generalization which can be readily identified. The protein-affected differences in the infectious episodes, whether the disease was of an acute or protracted nature, are differences which are evident only in the early stages and eventually are obliterated; eventually the animals all die or else die to the same frequency. In a word the claim for an effect on resistance is based on differences in survival time and not on frequency of survival, or survivorship. For precision and ease of detecting these facts it would be desirable that the Dubos-Schaedler experiments be presented in a statistically analyzed form as previously recommended (8). In view of the continuing experimentation, the statistical ambiguities may well be resolved to the satisfaction of all.

The Dubos-Schaedler experience is worth discussing further, however, on two counts: (a) what is known in the science of nutrition which suggests caution in interpreting experimental results as being due to "protein"; and (b) what is the relation of differences in survival time versus survivorship in the resistance problem.

The nutrition of a host animal is composed of a number of entities now listing in the order of 50 items. In weight alone, a constituent such as protein may occupy 20 per cent of the mass consumed, and its quantitative manipulation inevitably affects the proportions of others. This in turn, demands the introduction of such devices as paired-feeding techniques, isocaloric substitutions, controlled gain, and the like. Nutritional studies in the past have revealed some of the phenomena attendant on reduction of the plane of protein intake, two of which conceivably have a bearing on the present discussion. Beginning in the late 1930's and continuing to 1950, E. B. Forbes and his collaborators (9) published a series of papers on the relation of protein intake to energy metabolism which documented the iconoclastic view that in otherwise adequate dietary intakes *reduction* of protein led to an *increase* in heat output per calorie ingested. The classic view had been that protein, in its so-called "specific dynamic action," increased heat output. It was difficult to see how reducing protein could increase heat production until it had been made clear that the classic view rested on data obtained by feeding single foodstuffs to fasting animals and that Forbes's experiments showed what was the case in an animal eating enough food for growth and maintenance. Forbes's view is particularly pertinent to the present discussion. If reduced protein shortens survival time of the infected animal, the heightened metabolism may be indeed the root of the matter. This interpretation would be consistent with the effects of such metabolic stimulants as thyroxine and dinitrophenol on survival time. The effects of endotoxin, in the same direction, may likewise be referable to its pharmacological action in producing hyperthermia.

A second consequence of reduced protein level under conditions of *ad libitum* feeding should be mentioned. Meyer (10) has recently analyzed the consequences of protein composition of the diet on body composition of rats and found that, consistent with Forbes, the extraneous calories an animal consumes as it attempts to compensate for low protein levels by eating more of the diet, is stored as fat. On 6 per cent casein, fat formed 93 per cent of the carcass gain; on 18 per cent casein, fat formed only 13 per cent of the carcass gain. Thus, by manipulating protein we end by changing the fat content of the study unit, the host. That increased fat can influence resistance adversely under some circumstances has been reported earlier (11).

As a final comment on this aspect of the Dubos-Schaedler experience with altered protein intake

levels, the question may be raised whether extending the range of protein intake *above* 20 per cent, say to 45 per cent, would have any further effect. Such an increased intake, for example, has divergent effects on the two related phenomena we have just been discussing. Heat production continues to fall in this event whereas on the other hand the proportion of carcass fat rises again from the low experienced at the 18 per cent protein level. What would be the effect on survival time in an infection experiment?

With the publication of their most recent paper (3), the Dubos-Schaedler experience has been given a new definition. The diminished survival time has been shown not to be referable to a change in the fate of the infecting bacteria, but to an altered responsiveness to bacterial toxin. By judicious combinations of gram-negative bacterial endotoxin with the dose of infecting bacteria the phenomenology has been shortened in time, the survival time differences now assuming the order of hours rather than days. The new experiences, however, are still embraced by the generalization with which we began, *viz.*, the claimed differences are differences in survival time and not in survivorship (survival frequency). We turn now to examine the meaning of the respective phenomenologies, survival time versus survivorship, as they bear on the general problem of resistance.

The semantics of the word "resistance" offers a wide field of study in itself, but without attempting any complete analysis it may be estimated that all are aware that many procedures have been advanced to fulfill our urge to measure this all-too-vague entity. A survey of the literature reveals, however, that two forms of measurement are the ones used most widely. These are reportable as mean survival time and the frequency of survivial, or survivorship, respectively. Operationally considered, the first reports the outcome of an infected host sample in terms of time, and the second, estimated when the infectious episode is well over and the state of surviving is in no further jeopardy, reports the outcome in terms of frequency. Now, it is most often the case that no sooner has either of these reports been rendered than the reporter has begun to refer to them, not in operational terms, but in terms of "resistance." The question must now be raised whether both of these reporters have equal and legitimate claim to transmute their experience into the concept. It is suggested that the answer to this question is "No," for the following reasons.

In table 1 are listed some of the properties of these two methods of measurement of host-manipulated effects in infectious disease. The table also indicates the appropriate references to the literature on which the categorical statements themselves are based. From this admittedly incomplete analysis one is tempted to conclude that experimentally arranged differences in mean survival time have a different base, as phenomena, than do differences in survivorship. Until the relationship between these two is better understood it would be wise to exercise extreme caution in any interpretations in terms of "resistance." A safe course would be to report the experiments in their operational terms and defer interpretation. Some of the conflicting statements in the literature may have their basis in the too ready use of the words of common discourse, of which "resistance" is a prime example.[1]

But if these are two distinct phenomenologies, and if as experimenters we must in one way or another deal with them, it does not necessarily follow that because they are separate they are equal. Indeed, of these two separate bodies of facts, one might say, as has been said, "All facts are not created equal." It follows that a value judgement is required of us as to which phenomenology is the more likely to have relevance for the problem of "resistance." The choice for the phenomenology of survivorship seems the more reasonable, for the following reason. When we emerge as survivors of, say, a childhood bout of measles, experience entitles us to congratulate ourselves as having joined *for the rest of our lives* the fraternity of "measles survivors." We have demonstrated enough resistance to measles to survive it. It may be suggested that our satisfaction would be far less if, in "resisting" measles, we merely prolonged our mean survival time, only to die of measles in the end. If we choose

[1] In the opening pages of the *Principia*, Newton found it convenient to use the word "resistance" in his definition of the all-important notion of inertia. Apparently the idea of "resistance" is so embedded in our common speech and in the relations of our muscles to a physical world, that Newton found it convenient to trade on it without defining it. Our use of it in infectious disease argues our unspoken supposition of "opposed force" and makes clear the motivation of the many searches for what is presupposed to exist.

TABLE 1

Phenomenological aspects of mean survival time versus survivorship as epistemic correlates of natural resistance

Phenomenon	Datum	
	Mean survival time differences	Survivorship differences
Statistical model	Parametric[a]	Nonparametric[a]
Pathogen kinetics in the host	Independent[b]	Dependent[c]
Genetic structure of pathogen population	Role unknown, probably unimportant[d]	Crucial role[e]
Degree of response to nutritional dose	No theoretical specification[f]	Increments, by probits, linearly related to log dose[g]

[a] Siegel (12).
[b] Dubos and Schaedler (3).
[c] Schneider and Zinder (13).
[d] Probably unimportant since survival time differences have emerged in various experiments, in various laboratories, and with various pathogens without the apparent necessity of specification of the genetic structure of the pathogen population, *i.e.*, whether homogeneous with respect to virulence or not.
[e] Schneider (14).
[f] None has been found in the literature.
[g] Schneider (15).

survivorship free of all subsequent jeopardy, as the phenomenon worth striving for, then a further benefit will follow. This further benefit will be the impulse to be less than satisfied with those models of infectious disease in which our host manipulations result only in mean survival time differences and not in survivorship differences. If we press on with such a model we may well neglect the latter and miss the greater prize. But, being thus alerted, it may prove more rewarding in the end to reinspect such models and, in the light of the analyzed components of successful survivorship models, change them. Such a course, for example, might prove rewarding in the presently used models of experimental tuberculosis.

As a final comment on this ambiguity in the relationship of mean survival time versus survivorship it may be useful to entertain the view that the situation is analogous to one well recognized in chemistry. Consider the relation of a catalyst to the equilibrium constant of a chemical reaction. It is well established that a catalyst will affect the kinetics of a reaction but is powerless to change the equilibrium. If, therefore, at some early time the positively catalyzed reaction is compared with an uncatalyzed one, differences will be found in the relative concentrations of the reactants in the two systems. These differences, however, are obliterated as the reaction approaches equilibrium and at some finite later time, when equilibrium has been achieved in both systems, the differences have disappeared.

Just so in the phenomena of infectious disease an equilibrium is finally attained between host and pathogen. By analogy, differences in the kinetics of this host-pathogen reaction can yield temporal differences in survival (differences in mean survival time) but at the equilibrium point survival frequencies (survivorship) will be the same. It seems to the writer that it is on this equilibrium, *i.e.*, survivorship, that we should concentrate our aims and that differences in survivorship should be our goal.

Differences in survivorship, on the record, are achievable and their manipulation by means of host nutrition now makes it possible, as a next stage in inquiry, to ask something more. This question might be phrased as follows: If survivorship can be increased by increasing some dietary component, what is the predictable magnitude of this increase in survivorship as related to the increased nutritional dose? The theoretical and practical implications of an answer to this question are obvious enough not to be labored here.

A precise answer is now available in the instance of the mouse salmonellosis model, as indicated by table 1, lower right. The nutritional dose relationship to survivorship response is pre-

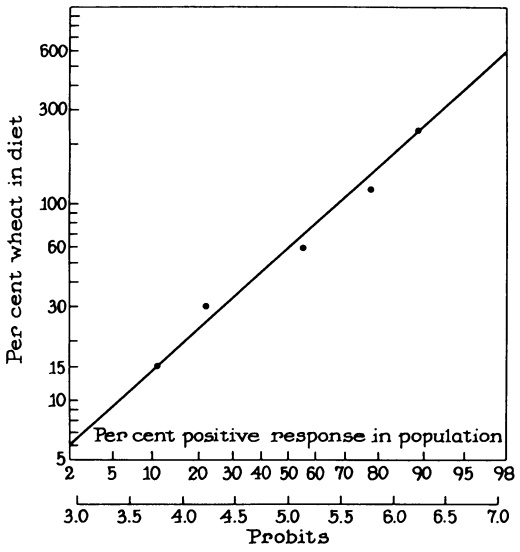

Figure 1. Increased survivorship in mouse salmonellosis as a function of dietary concentration of the salmonellosis resistance factor found in certain wheats.

sented in detail in figure 1. (Details of the investigations leading to the suitably transformed data of figure 1 have been presented in reference 15.) Set forth in this way we can see that increases in survivorship achieved by *logarithmic* increases of the dietary concentration of an unknown entity recognizable in certain wheats, are linearly related in terms of a statistical parameter of the host population, *i.e.*, the probit.[2] It should be pointed out that the probit is an attribute of a normally distributed population and the linearity of the transformation of figure 1 is in part proof that the mouse population used in these experiments in indeed "normally distributed." This probably is referable to the panmictic breeding system devised for this mouse stock some 15 years ago (14) and maintained since.

[2] It should be pointed out that differences in survivorship are describable on the basis of nonparametric statistics. When we are no longer satisfied with the mere *fact* of a difference but ask "How much is the difference?", we are forced to examine the responding host population in terms of a more detailed description. The problem of *magnitude* of survivorship differences thus leads into parametric statistics with its increased load of assumptions and the need for verification of these assumptions. For a more detailed discussion see Siegel (12).

Many relationships can be deduced from figure 1 but only a few can be dealt with here. One outstanding feature is that this nutritional manipulation of survivorship in mouse salmonellosis is "open ended" and has no formal limits. The dietary requirement for survivorship, in these conditions and with hosts in panmixia, can be stated only upon stipulation of the size of the host population and the survivorship frequency desired. Theoretically, the nutritional dose for 100 per cent survivorship is infinite, but this would be true only for populations of infinite size. Since finite populations are usually dealt with it follows that the nutritional dose will be finite, too.

One other lesson drawn from figure 1 has significance in illuminating the curious past history of investigations in nutrition and infection. The nutritionist must begin his search of the natural world by manipulating foodstuffs as he finds them. If, as it finally turns out, to achieve marked differences in survivorship his supplements must be added on a scale that exceeds the confines of the displaceable constituents of his basal diet, it can readily be appreciated that his early studies will be attended by many technical difficulties. For it is not always given that a rich source of what he is after will be found in some special foodstuff. Lacking such a source he must chemically concentrate the all-important factor. To do this he needs an assay, and he must begin with an assay which is not uniformly sensitive in terms of response in increments of survivorship because of the facts so clearly apparent in figure 1. Is it any wonder that progress has been slow? Is it any wonder that the field has been littered with claims, denials, and counterclaims? But is it not also true that a prize, which we have been at some pains in this conference to convince ourselves is of some importance, that such a prize cannot be meanly won?

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